Store at -20°C

#8579

Hippo Signaling Antibody Sampler Kit

☑ 1 Kit
(9 x 20 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 06/16

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-YAP (Ser397) (D1E7Y) Rabbit mAb	13619	20 µl	75 kDa	Rabbit IgG
LATS1 (C66B5) Rabbit mAb	3477	20 µl	140 kDa	Rabbit IgG
Phospho-MOB1 (Thr35) (D2F10) Rabbit mAb	8699	20 µl	24 kDa	Rabbit IgG
MOB1 (E1N9D) Rabbit mAb	13730	20 µl	25 kDa	Rabbit IgG
Mst1 Antibody	3682	20 µl	59 kDa	Rabbit IgG
Mst2 Antibody	3952	20 µl	60 kDa	Rabbit IgG
SAV1 (D6M6X) Rabbit mAb	13301	20 µl	45 kDa	Rabbit IgG
Phospho-YAP (Ser127) (D9W2I) Rabbit mAb	13008	20 µl	65-75 kDa	Rabbit IgG
YAP/TAZ (D24E4) Rabbit mAb	8418	20 µl	50,70 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Description: The Hippo Signaling Antibody Sampler Kit provides an economical means of detecting target proteins of the Hippo signaling pathway. The kit includes enough antibody to perform two western blots with each primary antibody.

Background: Hippo signaling is an evolutionarily conserved pathway that controls cell proliferation, apoptosis, and organ size in response to changing cell density levels (1,2). At relative low cell density, transcription co-activators YAP and TAZ bind transcription factors to induce expression of genes that favor cell growth and proliferation. As cell density increases, interaction between membrane-bound upstream hippo pathway regulators trigger activation of cytoplasmic kinases Mst1/2 and LATS1/2. Activated Mst kinase (the eponymous Hippo in *Drosophila*) associates with the adaptor Sav1 and activates the downstream LATS kinase, which phosphorylates YAP and TAZ (3).

Specificity/Sensitivity: Phospho-YAP (Ser397) Rabbit mAb recognizes endogenous levels of YAP protein only when phosphorylated at Ser397. This residue corresponds to Ser381 of YAP isoform 2, as reported by Zhao, B. et al. (2010) Genes Dev 24, 72-85 (9). Phospho-YAP (Ser127) (D9W2I) Rabbit mAb recognizes endogenous levels of YAP protein only when phosphorylated at Ser127. YAP/TAZ (D24E4) Rabbit mAb recognizes endogenous levels of total YAP and TAZ proteins. LATS1 (C66B5) Rabbit mAb recognizes endogenous levels of total LATS1 protein. Phospho-MOB1 (Thr35) (D2F10) Rabbit mAb recognizes endogenous levels of MOB1 protein only when phosphorylated at Thr35. MOB1 (E1N9D) Rabbit mAb recognizes endogenous levels of total MOB1 protein. This antibody detects both MOB1A and MOB1B. Mst1 Antibody recognizes endogenous levels of total Mst1 protein. This antibody does not cross-react with Mst2-4. Mst2 Antibody recognizes endogenous levels of total Mst2 protein. This antibody does not cross-react with Mst1, Mst3, or Mst4. SAV1 (D6M6X) Rabbit mAb recognizes endogenous levels of total SAV1 protein.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues near the amino terminus of human Mst1 protein, or residues near the amino terminus of human Mst2 protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues near the amino terminus of human MOB1A protein, residues surrounding Gly180 of human LATS1 protein, residues near the carboxy terminus of human SAV1 protein, or residues surrounding Asp362 of human TAZ protein. Phospho-specific monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr35 of human MOB1 protein, residues surrounding Ser397 of human YAP protein isoform 1, or residues surrounding Ser127 of human YAP protein.

Background References:

(1) McNeill, H. and Woodgett, J.R. (2010) *Nat Rev Mol Cell Biol* 11, 404-13.

(2) Zeng, Q. and Hong, W. (2008) *Cancer Cell* 13, 188-92.

(3) Zhao, B. et al. (2007) *Genes Dev* 21, 2747-61.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#8579

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.